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DECOMPOSITION BY PLANKTONIC AND ATTACHED MICROORGANISMS IMPROVES CHEMICAL FATE MODELS

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Abstract—Terms for decomposition by both suspended and attached microorganisms were included in a model to calculate the fate of chemicals in aquatic systems. This model differs from previous models primarily by the inclusion of a term for decomposition by aufwuchs in addition to a term for decomposition by planktonic microorganisms. Decomposition by aufwuchs is represented as occurring at a rate limited by the serial processes of transport to a planar surface and decomposition at the surface. Two sets of experiments were conducted using replicate laboratory microcosms, each maintained with six different environments. The predictive capability was good, as indicated by the absence of statistically detectable differences between predictions and laboratory measurements. In making the predictions, a set of parameters describing the characteristics of the chemical was required. Included in these parameters were the rate coefficients for the two microbial decomposition terms. With both microbial decomposition terms included, the fate model accounted satisfactorily for observed methyl parathion concentrations using only a single, environment-independent rate coefficient for each term.

Keywords—Chemical fate models Exposure assessment Microbial decomposition Aufwuchs decomposition Model testing

INTRODUCTION

An ability to predict the behavior of chemicals in the environment is essential to effective regulation of the use and disposal of those chemicals. Recognition of this need has led to the development of several models for evaluation of the fate of aquatic pollutants [1-6]. In these models, it is postulated that a small set of processes, each involving one or more environmental factors, is responsible for the fate of an organic chemical in the aquatic environment. Existing models of chemical fate in aquatic environments include terms for bulk transport, volatilization, photolysis, hydrolysis, microbial decomposition and possibly other processes. In these models, microbial decomposition is usually represented by a first-order, second-order or a rectangular hyperbolic term. Previous models have not accounted for transport to and into surficial microbial colonies.

Second-order kinetics assumes that the rate of chemical decomposition is proportional to the concentration of the chemical and to the intensity of the reactant present in the local environment. A process, however, cannot be fully represented by a second-order model when factors other than probability of encounter control the reaction rate. For example, under appropriate conditions, dense colonies of organisms attached to surfaces can carry out decomposition of chemicals as rapidly as the chemical is transported to the surface. Here, the organisms are in excess and can be ignored in the net process description, which need consider only the transport process. Where significant microbial decomposition is carried out both by planktonic single cells and by surficial colonies, terms for both processes are needed to describe the overall process. We included both processes in a chemical fate model and tested its capability to represent chemical disappearance when both processes were operating.

The basic idea of the experiments was to introduce a pollutant chemical into the environment and measure its fate for comparison with predictions. Exposure analysis models comprised of component models for each process known to be potentially significant should be able to predict environmental concentrations of chemicals. This capability, naturally, depends heavily on the accu-

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racy of the input values (loading rates, values of the chemical properties and the fidelity with which the target environment is described). In testing a model, the input values must be known accurately to permit sound conclusions. Knowing the input values accurately, any failure to obtain accurate predictions must be attributed to model inadequacy. In testing this model, fidelity of the microbial decomposition component models was of primary concern. To make these tests, we used replicate laboratory microcosms operated to achieve several different environments, so as to require as much breadth of predictive capability as possible in the laboratory setting. We made predictions based on a model with the process terms of the Exposure Analysis Modeling System (EXAMS) plus a term for decomposition by aufwuchs. Predictions were made with parameters selected (Table 1) to represent the conditions in the laboratory environments and with the loading rate set the same as the actual rate used in the experiments.

For decomposition by suspended microorganisms where transport is not limiting, we used a simple second-order relationship:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -k_s CB \tag{1}$$

where C is the bulk concentration of methyl parathion (MP), B is microbial population density and k_s is the second-order rate coefficient.

To obtain the expression[1] that includes the influence on rate of disappearance of chemical by both transport to the colony's surface and decomposition by the colony, we made the simplifying assumption that the colony is a planar surface on which chemical decomposition occurs. Chemical concentration at the surface is the steady-state concentration that balances decomposition rate and transport to the surface. Disappearance rate from water is expressed as the mass flux from water across a unit area of surface multiplied by the specific area of the microbially colonized surface:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = j\frac{S}{V} \tag{2}$$

where j is the mass flux from the water to the surface, S is the area of the microbially colonized surface and V is the water volume.

Transport to the surface is driven by the chemical gradient between bulk water and surface:

$$j_T = -k_T(C - C_C) \tag{3}$$

where j_T is the mass flux due to transport and C_C is the chemical concentration at the colony's surface.

As in bulk water, decomposition at the colony's surface is assumed to follow second-order kinetics. Therefore, the flux due to decomposition is

$$j_C = -k_C B_C C_C \tag{4}$$

where B_C is cell density of the colony and k_C is the second-order rate coefficient. At the steady-state flux, $j = j_T = j_C$.

Solving for C_C after equating Equations 3 and 4, resubstituting into Equation 4 and simplifying gives

$$j = -\frac{C}{k_T^{-1} + k_C^{-1}B^{-1}}$$

Further substitution into Equation 2 gives the expression for loss of chemical from water via decomposition by attached microorganisms:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -\frac{SC}{V(k_T^{-1} + k_C^{-1}B^{-1})} \tag{5}$$

Net disappearance is the sum of Equations 1 and 5.

We did not measure k_T, k_C and B separately, but instead measured the net of the term in the denominator in separate experiments conducted at a similar Reynolds number as that of the continuously stirred tank reactors (CSTRs) (see Materials and Methods). Decomposition by aufwuchs is conceived as being potentially limited by either transport to the surface of the colony or by the rate of decomposition by the colony. Strictly, the equation represents the colony's surface as a plane to which chemicals move and on which chemicals disappear. The rate of disappearance across the surface is taken to be proportional to the density of organisms on the surface. More complex models of this process incorporating biofilm thickness with chemical decomposition throughout the biofilm have been used (for example, see ref. 7). We considered their level of complexity to be beyond our present needs.

[1]This derivation of the expression representing decomposition by surficial microbial colonies was obtained by V. Borzilov, Inst. Experimental Meteorology, Obninsk, U.S.S.R., 249020, in cooperation with R. Lassiter in a joint research project conducted at the Athens Environmental Research Laboratory, U.S. Environmental Protection Agency, Athens, Georgia, in August 1983 under joint U.S.-U.S.S.R. Project 02.03-31, "Forms and Mechanisms by which Pesticides and Chemicals are Transported."

Table 1. Computational parameters

Parameter	Units	Value	Data source
CSTR volume	L	240	Measured
Flow	L h^{-1}	21	Measured
Colonized area	cm^2	3×10^4	Measured
Temperature	°C	20	Measured
Rate constants (Pseudo first-order)			
Neutral hydrolysis	h^{-1}	1.7×10^{-4}	[2]
Alkaline hydrolysis, k_{OH}	$M^{-1} h^{-1}$	19.3	[2]
(k_{OH} [OH^-])	(h^{-1})	(approx. 1×10^{-4})	
Suspended bacteria, k_B	ml $CFU^{-1} h^{-1}$	4.6×10^{-8}	[2]
(k_B CFU ml^{-1})	(h^{-1})	(approx. 2×10^{-3})	
Attached bacteria, k_A	L $cm^{-2} h^{-1}$	9.2×10^{-5}	[10]
(k_A cm^2 ml^{-1})	(h^{-1})	(approx. 1×10^{-2})	
Photolysis	h^{-1}	2×10^{-5}	EXAMS simulation
Volatility	h^{-1}	6×10^{-7}	EXAMS simulation

MATERIALS AND METHODS

Holm et al. [8,9] described the chemical and biological composition and the behavior of the laboratory ecosystems, as well as the general behavior of methyl parathion in the test systems. The experimental procedures and descriptions, therefore, are given here only briefly. Two laboratory experiments were carried out: experiment 1 ran from December 1978 to May 1979, experiment 2 from June to September 1979. These studies were carried out in the aquatic ecosystem simulator (AEcoS), a controlled environment chamber housing a flume (19.5 m long, 46 cm wide, and 61 cm deep with a U-shaped cross section). The Plexiglas flume, lined with 4 mil (0.004 in.) Teflon film, was divided into two parts (A and B), each subdivided into eight 240-liter compartments. Each compartment had an outlet weir and an axially mounted mixing device. The effluent from an upstream compartment plus any additional chemicals required by an experiment were the influent for the next downstream compartment. The compartments are referred to as CSTRs (continuously stirred tank reactors). The test chemical MP was introduced into the upstream CSTR at a concentration of 50 $\mu g\ L^{-1}$.

Water, supplied by a deep well, was treated by reverse osmosis and deionization before introduction into the CSTRs. In these experiments, water flow rate and toxicant introduction rate were controlled using gravity flow from an all-glass, constant-head distribution system. Water temperature was maintained at 20°C with about 1.5°C day-to-night fluctuation. Water flow rate was maintained at 500 liters per day, providing a mean residence time of 12 h in each compartment. Dissolved chemicals in the influent water were controlled by continuously pumping chemical concentrate to selected compartments.

Bottom sediments, contained in stainless steel trays, were added to selected compartments. Stainless steel wire mesh baskets were buried in the sediments for sampling. Light intensity at the water surface was maintained at approximately 230 lux by a bank of Vita Lite fluorescent bulbs. The photoperiod was 12 h long, including ramp function light/dark transitions at the beginning and end of the photoperiod. Each compartment received different chemical inputs to select for different ecosystems. Locally collected organisms were introduced initially into compartments 3 through 8, with repeated introductions once weekly thereafter.

Calculations of expected concentrations in the laboratory systems were made using measures of the environmental characteristics obtained from each compartment, properties of the chemical obtained from separate experiments and loads as introduced or measured at the influent weir of a particular CSTR. As noted, EXAMS [6] was used for some of the computations, particularly calculations of photolysis rate and volatilization; for most of the other calculations a spreadsheet program was used. For the calculations, it was assumed that a steady state existed between chemical import and decomposition within the CSTR. We used a value (4.6×10^{-8} ml $CFU^{-1} h^{-1}$) for the second-order rate coefficient for decomposition by planktonic microorganisms that was obtained by Smith et al. [2] using collected organisms. For decomposition by aufwuchs, we used a value (9.2×10^{-5} L $cm^{-2} h^{-1}$) obtained by Lewis et al. [10]. Lewis et al. obtained these values in experiments carried out at approximately the same

Reynolds number as for the laboratory test systems and, therefore, the values represented the net rate coefficient resulting from transport and surficial decomposition.

For statistical analyses, experimental observations of MP concentration were paired with corresponding predictions. As noted above, two laboratory experiments were carried out. To obtain the model estimates corresponding to the observed data, we used the same rate coefficients for both experiments and for all CSTRs. The rates were calculated for each unit (experiment, CSTR, date) using input values describing conditions in that unit (pH, attached and suspended biomass, light level, etc.).

Rationale for data analysis

In analyzing our data to determine agreement of predicted with observed values, we used covariance analysis with predicted concentration being tested as the covariate. This approach allowed us to consider the possible association of model failure with factors (date, rep and CSTR) of the experiment. When there is total agreement between observed and predicted values, the regression line of observed on predicted values will have unit slope and zero intecept and no significant effects will remain after adjustment for the covariate. When predicted and observed values differ, however, the lack of agreement ideally will be attributable to a source identified by the significance tests. The experiments were replicated to test more properly the performance of the model on an experiment basis. In each experiment, data were collected during several time periods to test model performance with increasing system maturity. We expected no effects due to time because we operated the systems for several weeks prior to the experiments to attempt to achieve steady-state conditions.

Generally, our a priori rationale was that (a) significant effects due to rep would indicate inconsistent predictive capability due to uncontrolled factors associated with replicate blocks, (b) significant effects due to date would indicate inconsistent predictive capability due to factors associated with temporal effects, (c) significant deviation from unit slope would indicate failure of the model to perform uniformly over the range of concentrations, and (d) significant effects due to CSTRs, after adjustment for the regression, would indicate model failure to predict equally well for all environments.

The first two would provide grounds for further work to improve the methodology for operating laboratory ecosystems, as well as for additional research in related ecosystem theory, and could provide grounds for further work to determine why the model failed to perform uniformly. The latter possibility would provide grounds for pursuing attempts at model improvement.

Prior to analysis and interpretation of an experiment, criteria for rejection and acceptance are needed. These criteria emphasize the purpose of the test, make explicit what is rejected if significance is found and provide a basis for determining whether experimental results are appropriate for the test.

We wish to determine whether the model is capable of predictions that can be of aid in regulating chemicals that will be released into the environment. It is of more value to regulatory decision making to be able to accurately predict chemical behavior for common situations than for exceptional situations. It is both acceptable and expected that the model will fail to predict the fate of the chemical in an exceptional environment. Although EXAMS and related models are theoretically based and, therefore, are not limited in their range of predictability in the way that empirical models are, their range is not limitless. On the other hand, the model clearly should be able to predict chemical behavior under widely differing but stable environmental conditions.

We screened our experimental data set for occurrences of extreme data values and found some instances of data that were inappropriate for our tests. In the development of experimental ecosystems from clean container to sustained community, both gradual and sudden changes occur. One typical and often repeated, sudden change is a bloom of suspended microorganisms. Many such blooms were observed, most being small and short-lived. We ignored these, accepting them as probably not entirely abnormal, even in well-established natural systems, and therefore as contributors to natural unexplained variation. One occurrence, however, was extreme and could scarcely be ignored in light of our intent to test the capability of the model to predict chemical concentrations under stable conditions. A bloom of microorganisms occurred on the last sampling date of experiment 2 of such magnitude that we considered the situation to be anomalous, and, therefore, we dropped all data for this date from the analysis. The rationale for this decision, however, goes beyond simply dropping from consideration portions of the experiment that appear to be anoma-

lous. Our predictions are based partly on the use of bacterial cell counts, without discriminating between organisms that are actively decomposing the test compound and those that are not. This approach assumes a stable relationship between densities of component populations of the target system. To the degree that this assumption is inaccurate, predictions will be inaccurate. We regard bloom situations as transient and unlikely to maintain the same relationships among populations that exist during more stable periods. We rejected, therefore, the data from the large bloom because it represented an exceptional rather than a common situation.

RESULTS

As a consequence of the experimental setup involving serially connected CSTRs, magnitudes of mean MP concentration decreased downstream. Predicted concentrations were functions of system characteristics measured independently on the various CSTRs and therefore were subject to random variation. We treated predicted concentration as a covariate, tested for possible replication (rep) and time (date) effects, and examined residuals for systematic variation in relation to the CSTRs.

Ordinarily, the effects due to CSTRs would be incorporated into the covariance analysis so that, after adjustment for the covariate, tests on differences among CSTRs would reflect variation in the fidelity with which predictions matched observed values for the different environments. The predicted values, however, based on measurements of system characteristics, vary by approximately the same magnitude as do the observations. This situation precludes accurate estimation of slope when using the common within-CSTR pooled estimate, because the concentration range within each CSTR, relative to the uncontrolled variation, is quite restricted (see ref. [11] for a detailed discussion). Fortunately, the ranges of values within the different CSTRs are substantially disjoint, and collectively they span a range that is large compared with the errors associated with either the dependent variable (observed concentrations) or the covariate (predicted concentrations). The effective increase in range beyond that present within any CSTR permits a rather precise estimate of the linear relationship to be obtained when the regression is fitted over all CSTRs. Preliminary tests did not reveal heterogeneity of slope for the rep or date classes, so we used a slope estimate obtained by pooling over the rep and date combinations. Following this procedure, we were able to examine residuals for identifiable patterns in the values after adjustment for the covariate (i.e., after accounting for the relationship between observed and predicted concentrations).

Table 2 contains the analysis of covariance for the two experiments separately. In this analysis, emphasis is on the relationship between predicted and observed values, taking into account blocks (rep-date combinations) as possible experiment-related sources of variation. There was no evidence that the relationship between predicted and observed concentrations differed from a linear one, with unit slope and zero intercept. In neither experiment was there a significant effect attributable to date.

The pooled slope estimates for the two experiments are given in Table 2, along with the standard errors of the estimates. In addition, the lines labeled "Deviation from unit slope" in the tables denote significance tests corresponding to the hypothesis that the true value of the slope is unity. In neither case is this hypothesis rejected. ("Pr > F" represents the probability of obtaining a more extreme value of the observed F statistic.) For a particular rep-date combination we have a set of observation–prediction pairs, one pair from each CSTR. Thus a regression of observed values on predicted can be obtained for each rep-date combination. Individual intercept estimates, corresponding to the specific rep-date pairs and fitted with a common slope, are listed along with their standard errors. For each experiment, the hypothesis that all intercepts are zero can be tested jointly. The line labeled "Zero intercepts" contains the F test of this hypothesis. In experiment 1, there is no evidence that leads to the rejection of this hypothesis. Similarly, for experiment 2, at the 0.05 level of significance, the hypothesis of zero intercepts is not rejected.

The relationship between predicted and observed values is considered over the range of magnitude occurring in the whole set of environments (CSTRs). In this analysis, we do not make specific reference to the CSTRs. It is recognized, however, that either failure of the relationship to be of the expected zero intercept and unit slope, or for there to be a large variance of the slope, could result from the model's inability to make accurate predictions for conditions in one or more CSTRs. Therefore, additional analysis of variance for CSTR effect after adjustment for regression was carried out. These results are given in Table 3. In neither analysis was there evidence for failure of the model associated with CSTR.

Table 2. Tests of hypotheses and analysis of covariance

Source	df	SS	F	Pr > F	Estimates: Rep	Date	Intercept ± SE
Experiment 1							
Zero intercepts	8	73.74	1.49	0.203	A	1	0.74 ± 1.27
					B	1	−1.00 ± 1.35
Regression	1	1,301.01			A	2	0.48 ± 1.48
Due to unit slope	—	1,283.39			B	2	0.29 ± 1.43
Deviation from unit slope	1	17.62	2.86	0.102	A	3	1.52 ± 1.71
Blocks, adjusted	7				B	3	−2.68 ± 1.43
Rep, adjusted	1	39.23	6.36	0.017	A	4	−0.13 ± 1.35
Date, adjusted	3	11.20	<1		B	4	−1.94 ± 1.39
Rep × Date, adjusted	3	19.90					
Deviation from regression	29*	178.96			Slope		0.896 ± 0.06
Experiment 2							
Zero intercepts	6	275.67	1.83	0.127	A	1	1.82 ± 3.37
					B	1	3.01 ± 2.75
Regression	1	2,506.56			A	2	5.57 ± 2.83
Due to unit slope	—	2,503.79			B	2	−1.14 ± 3.36
Deviation from unit slope	1	2.77	<1		A	3	3.99 ± 3.02
Blocks, adjusted	5				B	3	−1.16 ± 3.20
Rep, adjusted	1	113.83	4.55	0.042			
Date, adjusted	2	6.67	<1				
Rep × Date, adjusted	2	91.55					
Deviation from regression	29	726.12			Slope		1.034 ± 0.103

*In experiment 1, there were two missing observations, so the degrees of freedom were adjusted accordingly.

Despite finding no model inadequacy associated with CSTR, additional analyses were performed (Table 4) using Duncan's multiple range test on residuals (after adjustment for regression) to determine whether there were failures of the model for specific CSTRs. These analyses are arranged by date within the experiments. No evidence exists for model failure in these analyses, but a curious tendency for ordering of the residual means is evident. The ordering is caused by predictions in one CSTR series being very poor, such that differences between highly ordered observations and nearly random predictions are themselves highly ordered. The poor predictions derive from highly fluctuating counts for suspended bacteria, instances in which transient changes were occurring in the biotic community similar to (but less extreme than) the instance already discussed for which we discarded the data.

DISCUSSION

When we used the model without a separate term for decomposition by microbially colonized surfaces, we found that the model was capable of representing the observations in each experiment only if separate biological decomposition rate coefficients were used for each experiment. This was an unsatisfactory result, because it gave grounds for rejection of the model for practical use without providing direction for improvement. In practice, the model is of major benefit only if situation-specific information requirements are minimal.

The demonstration that decomposition of toxic chemicals by surface-attached organisms is well represented by the use of the colonized surface area and a mixing parameter [12] provided the information needed to proceed beyond this earlier analysis. This information essentially provided a new model for testing, including two terms for microbial decomposition by forms attached to surfaces and by planktonic forms. Tables 5 and 6 give measured values and predictions using the sum of disappearance rate terms for both attached and suspended microorganisms and other data relevant to disappearance of the chemical. For decomposition by planktonic organisms, we used the second-order rate constant obtained by Smith et al. [2]. For decomposition by aufwuchs we used the rate coefficient based on specific surface area obtained by Lewis et al. [10]. No parameter values were estimated in conjunction with these experi-

Table 3. Tests for CSTR variation after adjustment for pooled regression

Adjusted for regression: Source	df	SS	F	Pr > F
Experiment 1				
CSTR	4	40.24	2.73	0.15
Block × CSTR	33	138.71		
Rep × CSTR	5	18.42		
Date × CSTR	15	54.88	<1	
Residual	13*	65.41		
CSTR (Date 1)	4	23.50	2.97	0.158
CSTR (Date 2)	4	10.51	<1	
CSTR (Date 3)	4	5.58	<1	
CSTR (Date 4)	4	55.54	1.77	0.297
Rep × CSTR (Date 1)	4	7.91		
Rep × CSTR (Date 2)	3*	16.03		
Rep × CSTR (Date 3)	3*	28.51		
Rep × CSTR (Date 4)	4	31.39		
Experiment 2				
CSTR	5	219.85	1.74	0.259
Block × CSTR	30	514.94		
Rep × CSTR	6	151.70		
Date × CSTR	12	157.12	<1	
Residual	12	206.12		
CSTR (Date 1)	5	58.45	<1	
CSTR (Date 2)	5	169.33	1.79	0.269
CSTR (Date 3)	5	149.19	<1	
Rep × CSTR (Date 1)	5	64.45		
Rep × CSTR (Date 2)	5	94.85		
Rep × CSTR (Date 3)	5	198.52		

*In experiment 1, there were two missing observations, so the degrees of freedom were adjusted accordingly.

ments. Considering the independence of parameter values from the experimental situation, the absence of evidence for rejection of the model as a good predictor supports its acceptance for use as an exploratory assessment tool.

In Table 2, rep adjusted for regression is significant at the 0.05 probability level for experiments. There is not a significant effect of date, and, therefore, we find no direct evidence of temporal variation in predictability. In the analyses for effects associated with CSTR (Table 3), we found no evidence that the model was inadequate as a function of CSTR overall or as a function of CSTR within date. In view of the lack of significance for date or CSTR, we attribute the significance of rep to a lack of functional uniformity in the experimental systems. Thus, although we found possible grounds on which to base further efforts to carry out replicate experiments, we found no evidence against the capability of the model to predict accurately in this experimental situation. This successful test of the component models of EXAMS and similar exposure analysis models is significant in two ways. First is the finding that the model is capable of calculating values for the concentration of chemical that cannot, as a body, be distinguished statistically from the body of observed data. This finding is, of course, only a small bit of information of which considerably more is needed on different chemicals and in diverse environments before one could confidently accept such models as being well tested.

The second way in which this test is significant is in regard to the method of testing per se. There are two aspects of the method of testing: the statistical approach and the experimental ecosystems. Typically, in hypothesis testing, the null hypothesis is formulated such that it is rejected if the experiment is successful. Here we have formulated our hypothesis in a manner more akin to the logic used in goodness-of-fit tests. Our null hypothesis is that of no difference between predicted and observed values (corresponding to zero intercept and slope of one in the covariance analysis). If our

Table 4. Separation of residual means

Date 1		Date 2		Date 3		Date 4	
C	*Y* residual	*C*	*Y* residual	*C*	*Y* residual	*C*	*Y* residual
Experiment 1							
6	1.98	5	1.30	8	0.85	6	3.22
4	0.79	4	1.20	4	0.75	4	1.20
8	0.14	6	−0.65	6	−0.09	8	0.41
7	−0.25	7	−0.86	5	−0.33	5	−0.98
5	−2.67	8	−0.99	7	−1.18	7	−3.85
Experiment 2							
3	2.76	3	5.54	5	6.68		
4	2.32	4	3.89	6	2.47		
5	0.66	5	0.96	4	−1.33		
6	−0.31	6	−2.83	3	−1.56		
7	−2.00	7	−3.00	7	−2.87		
8	−3.43	8	−4.57	8	−3.38		

Bars indicate means that do not differ significantly ($Pr \geq 0.95$).

experiment is successful, i.e., we have constructed and parameterized a model capable of predicting accurately, then we will not reject the null hypothesis. This formulation makes experimental design and performance of the experimental ecosystems especially important, because if the model is upheld by failure to reject the null hypothesis, it is the more strongly upheld the more critical is the test.

Tests that require the model to demonstrate predictive capability for previously unencountered situations are the sorts of tests likely to be productive in exposing model inadequacies. Although it is unlikely that the fundamental process representations are grossly in error, testing is a worthwhile activity. It is possible, for example, that the specific representation emphasizes the incorrect limiting factor. Representing microbial decomposition by surficial colonies by a simple second-order model is an example of such. As noted, microbial decomposition is fundamentally a second-order process occurring between the microbial enzyme and the chemical molecule undergoing decomposition. The rate-limiting step is determined by whether the organism is separately suspended or attached to a fully colonized surface, the whole of which is actively engaged in the decomposition process. The model need only represent the rate-limiting process, and, for decomposition by aufwuchs, the primary second-order model disappears. The uncertainty of whether this is done properly for all processes represented is due partly to uncertainty about the nature of the environment. Our tests have served to reduce this uncertainty to some degree, and to increase our confidence that our basis for modeling the fate of chemicals in aquatic environments is sound.

Specifically, these tests showed that the disappearance of MP, occurring predominantly via microbial decomposition, can be satisfactorily represented by a model with components for decomposition by both planktonic organisms and aufwuchs. There is no reason to expect that this result applies specifically to this compound only, and therefore the tests have improved generally the credibility of this type of modeling as a tool for regulatory use. The results do not apply uniformly, however, to all chemicals whose environmental behavior is of interest before they are released. For chemicals whose major decomposition pathways differ from that of MP, the tests will be of considerably less relevance. The major decomposition pathway for MP within our range of environmental conditions was biological, and here there may be an additional generality. The component model for decomposition by microbially colonized surfaces is applicable to all chemicals whose decomposition is limited by transport of the chemical to the surface. In particular, not only does the same model structure apply to all such chemicals, but the rate coefficient is the same regardless of chemical because the rate coefficient is the transport rate, solely a property of the mixing characteristics of the water body. Unfortunately, no means is available to estimate a transport coefficient to surficial microbial colonies as a function of observable characteristics of a water body.

Table 5. AEcoS methyl parathion (MP) experiment 1

CSTR	Date	pOH	Suspended bacteria (CFU/ml)	Attached bacteria (CFU/cm^2)	Observed MP concn. (ppb)	Predicted MP concn. (ppb)
A3	2/20/79	4.96	2.4e3	2.1e6	17.6	21.20
	3/05/79	5.89	8.0e4	1.1e6		
	3/27/79	5.6	1.1e4	5.7e6	29.0	
	4/10/79	4.05	1.1e5	5.3e6	25.9	23.89
A4	2/20/79	4.96	3.7e3	2.2e6	15.7	15.52
	3/05/79	5.89	6.9e4	2.5e6	24.2	
	3/27/79	5.6	4.4e3	3.2e6	24.8	25.60
	4/10/79	4.05	8.6e4	4.6e6	19.8	21.66
A5	2/20/79	4.94	1.6e4	8.8e6	10.9	13.54
	3/05/79	5.48	6.7e4	5.8e6	17.9	20.41
	3/27/79	5.47	1.0e4	5.0e6	28.2	
	4/10/79	4.33	1.0e5	2.8e6	15.2	16.32
A6	2/20/79	4.92	5.5e3	9.9e5	10.6	9.61
	3/05/79	5.27	5.5e4	5.4e6	15.2	15.46
	3/27/79	5.37	1.2e4	1.8e6	22.8	24.83
	4/10/79	5.33	1.3e5	4.1e6	12.6	12.71
A7	2/20/79	4.72	1.2e4	1.6e6	9.9	9.30
	3/05/79	5.11	6.8e4	2.1e6	12.1	13.04
	3/27/79	5.21	9.8e3	3.0e6	16.9	20.07
	4/10/79	5.12	4.1e4	6.7e6	8.3	10.94
A8	2/20/79	4.58	1.8e4	8.2e5	7.3	8.65
	3/05/79	4.99	4.7e4	1.0e6	9.2	10.47
	3/27/79	5.1	9.1e3	5.3e5	15.1	14.88
	4/10/79	4.98	3.2e4	1.3e6	5.1	7.23
B3	2/20/79	4.96	2.8e3	2.3e6	28.4	32.82
	3/05/79	5.98	9.7e4	8.9e6	26.6	26.35
	3/27/79	5.77	1.2e4	3.3e6	35.2	
	4/10/79	5.79	3.9e4	3.0e6	30.9	
B4	2/20/79	4.96	3.4e3	6.7e5	22.0	25.08
	3/05/79	5.98	1.1e5	7.6e6	22.1	22.44
	3/27/79	5.77	8.7e3	3.3e6	26.3	31.07
	4/10/79	5.79	3.1e4	3.6e6	24.1	27.00
B5	2/20/79	4.91	6.3e4	1.2e6	12.3	18.60
	3/05/79	5.92	9.3e4	1.6e6	20.3	18.47
	3/27/79	5.54	1.2e4	5.2e6	14.1	22.79
	4/10/79	5.43	3.4e4	4.4e6	13.9	20.67
B6	2/20/79	4.87	6.2e4	1.6e6	11.2	10.58
	3/05/79	5.86	6.3e4	3.2e6	13.8	17.49
	3/27/79	5.39	8.5e3	1.4e6	9.2	12.44
	4/10/79	5.24	5.5e4	3.4e6	13.9	12.01
B7	2/20/79	4.67	5.4e4	7.8e5	6.32	9.65
	3/05/79	5.70	4.8e4	2.1e6	9.4	11.96
	3/27/79	5.23	9.4e3	1.0e6	4.9	8.15
	4/10/79	5.03	4.6e4	4.1e6	2.5	12.04
B8	2/20/79	4.53	1.3e5	6.3e5	5.1	5.24
	3/05/79	5.58	3.8e4	8.9e5	6.2	8.12
	3/27/79	5.12	7.6e3	1.8e6	2.6	4.29
	4/10/79	4.89	3.8e4	1.8e6	2.0	2.17

n.n e n indicates *n.n* × 10^n.

CONCLUSIONS AND RECOMMENDATIONS

A model to calculate pollutant fate that includes terms for decomposition by both suspended microorganisms and attached microbial colonies was tested. The model was parameterized with a single, independently obtained set of chemical properties, including a single rate coefficient for each of the microbial decomposition terms. The model performed well over the range of conditions of the test. The tests indicated that predictions cannot be distinguished statistically from observations

Table 6. AEcoS methyl parathion (MP) experiment 2

CSTR	Date	pOH	Suspended bacteria (CFU/ml)	Attached bacteria (CFU/cm^2)	Observed MP concn. (ppb)	Predicted MP concn. (ppb)
A3	7/24/79	6.14	1.2e5	2.4e7	41.4	38.73
	8/07/79	5.42	1.5e6	8.1e7	39.3	22.98
	8/21/79	5.56	3.0e4	4.1e7	39.0	37.31
	9/24/79	6.01	5.2e6	1.3e8	44.1	11.28
A4	7/24/79	6.14	1.0e5	9.5e6	38.1	34.98
	8/07/79	5.42	1.0e6	3.0e7	36.0	23.57
	8/21/79	5.56	3.1e4	2.1e7	32.4	34.00
	9/24/79	6.01	5.5e6	6.8e7	40.5	10.81
A5	7/24/79	5.93	6.6e5	7.8e6	33.0	25.28
	8/07/79	5.62	1.1e6	3.0e7	27.3	20.62
	8/21/79	5.83	3.0e6	1.7e7	30.6	11.67
	9/24/79	5.96	3.6e6	8.7e7	33.0	13.05
A6	7/24/79	5.79	6.1e5	9.3e6	25.5	22.91
	8/07/79	6.0	8.4e5	2.1e8	20.4	17.53
	8/21/79	6.64	2.9e6	1.5e7	22.5	11.73
	9/24/79	5.92	8.1e5	4.5e7	27.9	21.40
A7	7/24/79	5.51	6.2e5	5.8e6	19.9	17.62
	8/07/79	5.82	3.5e5	9.0e6	16.5	15.59
	8/21/79	5.96	1.8e5	2.0e7	18.6	18.41
	9/24/79	6.04	1.2e6	5.0e7	21.0	16.00
A8	7/24/79	5.34	1.9e5	6.1e6	14.1	16.22
	8/07/79	5.69	1.7e5	9.2e6	11.7	13.57
	8/21/79	5.71	1.1e5	1.7e7	14.1	15.69
	9/24/79	6.2	5.4e5	2.0e7	17.1	14.96
B3	7/24/79	5.95	5.5e5	1.1e7	39.9	31.42
	8/07/79	5.22	3.0e4	1.7e7	38.7	38.41
	8/21/79	5.19	7.0e4	2.0e7	33.9	35.03
	9/24/79	5.81	6.3e6	4.4e7	40.3	10.07
B4	7/24/79	5.95	8.3e5	1.4e7	33.0	25.59
	8/07/79	5.22	3.0e4	1.8e7	34.8	33.80
	8/21/79	5.19	1.3e5	2.5e7	31.2	28.32
	9/24/79	5.81	3.9e6	5.0e7	34.4	12.78
B5	7/24/79	5.82	6.6e5	1.1e7	21.9	22.07
	8/07/79	5.32	2.9e5	2.6e7	27.9	26.75
	8/21/79	5.39	5.6e4	5.8e7	25.5	26.50
	9/24/79	5.88	2.2e7	1.3e8	27.6	2.70
B6	7/24/79	5.72	1.3e6	1.2e7	15.6	12.24
	8/07/79	5.44	1.5e5	6.7e6	20.7	23.15
	8/21/79	5.77	4.8e4	1.5e7	21.0	22.12
	9/24/79	5.97	1.8e7	2.1e7	23.1	2.69
B7	7/24/79	5.44	9.8e5	8.7e6	10.1	9.60
	8/07/79	5.26	3.5e4	4.8e6	17.4	18.04
	8/21/79	5.09	1.4e5	1.4e7	16.2	17.48
	9/24/79	6.09	5.0e6	4.2e7	21.0	6.32
B8	7/24/79	5.27	9.3e5	4.6e6	8.4	6.23
	8/07/79	5.13	5.7e4	3.2e6	14.1	14.97
	8/21/79	4.84	6.5e4	1.9e7	13.5	13.87
	9/24/79	6.25	2.3e7	4.2e7	17.4	1.59

n.nen indicates $n.n \times 10^n$.

made on a test system. Models of chemical fate in aquatic systems should include separate terms to represent decomposition by suspended and attached microbial decomposers. With both microbial decomposition terms present, we conclude that fate models, such as EXAMS, will provide useful estimates of chemical fate in a wider variety of aquatic environments than without. Additional testing is needed, however, for fate models that include both microbial decomposition terms. These tests should be made using other chemicals and a greater diversity of environments.

REFERENCES

1. **Lassiter, R.R., J.L. Malanchuk** and **G.L. Baughman.** 1976. Comparison of processes determining the fate of mercury in aquatic systems. *Proceedings*, EPA Conference on Environmental Modeling and Simulation. EPA-600/9-76-016. U.S. Environmental Protection Agency, Athens, GA, pp. 619-623.
2. **Smith, J., W.R. Mabey, N. Bohonos, B.R. Holt, S.S. Lee, T. -W Cou, D.C. Bomberger** and **T. Mill.** 1978. Environmental pathways of selected chemicals in freshwater systems. Part II: Laboratory studies. EPA-600/7-78-074. U.S. Environmental Protection Agency, Athens, GA.
3. **Lassiter, R.R.** and **J.L. Malanchuk.** 1978. A model evaluation of the relative importance of factors influencing the environmental behavior of mercury. In D.C. Adrian and I.L. Brisbin, Jr., eds., *Environmental Chemistry and Cycling Processes.* U.S. Department of Energy Conf-760429, Augusta, GA, pp. 182-195.
4. **Baughman, G.L.** and **R.R. Lassiter.** 1978. Prediction of environmental pollutant concentration. In J. Cairns, Jr., K.L. Dickson, and A.W. Maki, eds., *Estimating the Hazard of Chemical Substances to Aquatic Life.* ASTM Special Publ. 657. American Society for Testing and Materials, Philadelphia, PA, pp. 35-54.
5. **Neely, W.B.** 1981. *Chemicals in the Environment—Distribution, Transport, Fate, Analysis.* Marcel Dekker, Inc., New York, NY.
6. **Burns, L.A., D.M. Cline** and **R.R. Lassiter.** 1982. Exposure analysis modeling system (EXAMS): User manual and documentation. EPA-600/3-82-023. U.S. Environmental Protection Agency, Athens, GA.
7. **Rittman, B.E.** and **P.L. McCarty.** 1981. Substrate flux into biofilms of any thickness. *J. Environ. Eng. Div. A.S.C.E.* **107**:831-850.
8. **Holm, H.W., H.P. Kollig, L.M. Proctor** and **W.R. Payne, Jr.** 1982. Laboratory ecosystems for studying chemical fate: An evaluation using methyl parathion. EPA-600/3-82-020. U.S. Environmental Protection Agency, Athens, GA.
9. **Holm, H.W., H.P. Kollig, W.R. Payne, Jr.,** and **L.M. Proctor.** 1983. Fate of methyl parathion in aquatic channel microcosms. *Environ. Toxicol. Chem.* **2**:169-176.
10. **Lewis, D.L., R.P. Kellogg** and **H.W. Holm.** Comparison of microbial transformation rate coefficients of xenobiotic chemicals between field-collected and laboratory microcosm microbiota. In T.P. Boyle, ed., *Validation and Predictability of Laboratory Methods for the Assessment of Fate and Effects of Environmental Contaminants in Aquatic Ecosystems.* STP 865, ASTM, Philadelphia, PA.
11. **Huitema, B.E.** 1980. *The Analysis of Covariance and Alternatives.* John Wiley & Sons, New York, NY.
12. **Lewis, D.L., H.P. Kollig** and **T.L. Hall.** 1983. Predicting 2,4-dichlorophenoxyacetic acid ester transformation rates in periphyton-dominated ecosystems. *Appl. Environ. Microbiol.* **46**:146-151.

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